



## Tadpole assemblage in temporary ponds in southern Piauí, Brazil

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**Abstract.**—The ecological relationship between tadpoles and waterbodies, considering both biotic and abiotic factors, is poorly understood. This data gap is notable in South America, especially in xeric regions such as Northeastern Brazil. In this study, the distribution, abundance, and interactions between tadpole species were investigated by examining their spatial and seasonal patterns in a semiarid environment in Northeastern Brazil. The relationships between the species and their environments were assessed through the ecological descriptors of prevalence, mean intensity, mean abundance, Green's index, dominance ratio, and diversity. Forty-eight temporary ponds were sampled, tadpoles were found in 24 of them, and the 403 individual tadpoles collectively represented 12 species from four families (Hylidae, Leptodactylidae, Microhylidae, and Phyllomedusidae). These species presented random aggregation behavior without interspecific dependencies, and with only three dominant species. Of the abiotic variables, the hydroperiod had the greatest influence on larval behavior. There was no relationship between pond occupation and the physicochemical water properties based on the coefficients of determination ( $R^2$ ). The highest coefficient found was  $R^2 = 0.33$  (for parameters pH and species). However, water reduction permitted only the most advanced stages of development to remain in the pond. The successful occupation of xeric environments by frogs is directly related to their capacity to adapt to ephemeral waterbodies, in which the main limiting factor is water availability.

**Keywords.** Amphibian, anuran, Caatinga biome, ephemeral waterbodies, metamorphosis, xeric environment

**Citation:** Lima MSCS, Pederassi J, Souza CAS. 2022. Tadpole assemblage in temporary ponds in southern Piauí, Brazil. *Amphibian & Reptile Conservation* 16(1) [General Section]: 257–264 (e314).

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**Accepted:** 10 August 2020; **Published:** 29 July 2022

### Introduction

Biocenosis and assemblage are collective nouns referring to ecological communities that are formed by different species. Most tadpoles occupy an aquatic environment during their development from egg to adult. Many tadpole characteristics show plasticity, such as variations in the time of development according to abiotic factors (e.g., temperature, pH, dissolved oxygen, or water availability), or the timing of lung development or other changes that accelerate or decelerate the individual's ontogeny. This plasticity confirms that their morphological differences such as buccal apparatus, fin size, and caudal musculature, with their conspicuous hydrodynamics and gregarious or solitary behavior (that is generally related with the feeding mode), are

associated with the different ecological niches that they occupy (Altig and McDiarmid 1999; Tejedo et al. 2000; Gomez-Mestre and Tejedo 2002; Andrade et al. 2007). Thus, intrinsic and extrinsic factors can affect the way they occupy the aquatic environment (Fatorelli and Rocha 2008).

Several studies on tadpoles in Brazil include those examining their spatial distributions and patterns of diversity (Rossa-Feres 2014), taxonomic studies seeking to identify knowledge gaps in their descriptions (Bokermann 1967, 1968; Rossa-Feres and Nomura 2006; Proverte et al. 2011), studies on the reproduction and biology of *Lysapsus limellum* Cope, 1862 in the Pantanal (Prado and Uetanabaro 2000), observations on the dynamics of tadpole nests and embryos of *Boana faber* (Wied-Neuwied, 1821) by Martins (1993), and

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a description of *Boana wavrini* (Parker, 1936) tadpole nests by Martins and Moreira (1991). However, few studies have investigated the interactions between tadpoles and their environment. Likewise, studies on many other aspects of tadpoles are also scarce, such as the modelling of tadpole guilds (Fatorelli and Rocha 2008), the gregarious behavior of *Lithobates catesbeianus* (Shaw, 1802) tadpoles (São Pedro et al. 2008), and the history and perspectives on tadpoles in Brazil (Andrade et al. 2007). Although these studies are from more than a decade ago, the gaps in our knowledge regarding the taxonomy, diversity, and distribution of tadpoles in Brazil have barely advanced, and this area remains to be explored and better understood. In the northeast of Brazil, these gaps are highlighted by the complete absence of research in this field. This area is also of special interest due to the particular characteristics of its xeric biome, Caatinga, where the mean temperature remains above 30 °C during the year, and the rain is less than 1,400 mm and concentrated from November to February.

We hypothesize that the adaptations to this ephemeral optimal condition (i.e., the availability of water bodies) are the primary factors which determine the success of the species in this Brazilian xeric environment. Considering the emerging research on tadpoles, the knowledge gap regarding populations in the semi-arid region of Piauí, Brazil is profound. Therefore, this paper contributes to our understanding of the distribution, abundance, and interactions among tadpole species by identifying the patterns of dispersion (gregariousness) and determining how the extreme semi-arid conditions, primarily the short periods of available hydric resources, shape the development of the tadpoles. The results will improve our knowledge of tadpoles in the lentic environments of the semi-arid region in Northeastern Brazil.

### Materials and Methods

**Study area.** The study area consisted of a partially flooded site (10,500 m<sup>2</sup>) comprised of Carnauba palms, *Copernicia prunifera* (Mill.) H.E. Moore, and several aquatic weeds such as Ginger-leaf Morning-glory [*Ipomoea asarifolia* (Desr.) Roem. and Schult.], Baldhead False Buttonweed (*Spermacoce capitata* Ruiz and Pav.), and Valdivia Duckweed (*Lemna valdiviana* Phil.). The site was located on highway BR-343, near km marker 600 (6°54'34.1"S 43°09'37.7"W), in the Municipality of Floriano, State of Piauí, Brazil.

**Study units.** A total of 48 ponds were sampled, among which 24 had the presence of tadpoles, and they were used as the sample units. The other 24 ponds were excluded after three successive sweeps with plastic sieves failed to show the presence of any tadpoles. The data on pond characteristics are given in the section Hydroperiod and Abiotic Data below.

**Species sampling.** The species sampling method for comparing the ponds used the sum of the number of tadpoles captured during 15 uninterrupted minutes of sweeping by three researchers, one occupying the central region of the pond, while the other two occupied the borders of the pond. They walked side-by-side sieving the water from one side of the pond to the other along a north-south transect. Each researcher collected the tadpoles using a plastic sieve (280 mm × 160 mm × 70 mm, wire thickness 0.36 mm, and mesh size 10) attached to a 15 cm cable. The sampling was conducted for 29 consecutive days between November 5, 2017 and December 2, 2017. The collected tadpoles were placed in a 5-L plastic container with a diameter of 160 mm. In the lab, they were euthanized by immersion in a 5 g/L solution of benzocaine and fixed by immersion in 5% formalin before identification to the species level and the classification of their developmental stage according Gosner (1960).

### Hydroperiod and Abiotic Data

All 48 ponds were evaluated according to the parameters described below until the water disappeared completely, and these data were subjected to univariate linear regression to determine the retraction of the waterbody. For each pond, including the ponds that did not include any tadpoles, the temperature (°C), dissolved oxygen (mg L<sup>-1</sup>), and pH were measured. Substrate samples followed the ABNT/NBR (Associação Brasileira de Normas Técnicas/Norma Brasileira) 7181/82 standards and were classified by the Laboratory of Herpetology at the Federal University of Piauí (UFPI) in Brazil as either gravel ( $\phi > 2.0$  mm), sand ( $2.0 \text{ mm} \geq \phi > 0.05$  mm), silt ( $0.05 \text{ mm} \geq \phi \geq 0.002$  mm), or clay ( $\phi < 0.002$  mm). The ponds had an average depth of  $15 \pm 4.0$  cm as measured at three points in each direction. The first point was in the center of the pond and the other two points were equidistant between the center and the borders in both the north-south and east-west directions. The mean was considered as the depth parameter. The abiotic and species occupation data were analyzed using the R<sup>2</sup> coefficient of determination, to determine any interdependence between the variables.

**Ecological descriptors.** The prevalence, mean intensity, mean abundance, Green's index, and the value of importance were calculated following Legendre and Legendre (1998) and Zar (2010).

Prevalence ( $P$ ) was calculated as:

$$P = \frac{n}{N} \cdot 100$$

where  $n$  is the species found in a sample and  $N$  is the sum of the daily sample.

The Mean Intensity ( $MI$ ), or the magnitude of the proportion of the species, was calculated as:

$$MI = \frac{n}{\Sigma n}$$

where  $n$  is the number of individuals of a species in a sample and  $\Sigma n$  is the sum of the individuals of that species recorded among all samples.

Based on the above results, the Mean Abundance ( $MA$ ) was calculated as:

$$MA = MI \times P$$

The dispersion index, or Green's Index ( $GI$ ), was calculated by the variance/mean ratio as:

$$GI = \frac{\left[ \frac{S^2}{\bar{X}} \right]}{(n-1)}$$

where  $S^2$  is the sample variance,  $\bar{x}$  is the sample mean, and  $(n-1)$  is the degrees of freedom. When the  $GI$  value is close to 0, the dispersion is random; and when it is close to 1.0, the dispersion is maximally aggregated.

The value of importance ( $I$ ) was calculated as:

$$I_{j=(M_j) \left( \frac{A_j B_j}{\Sigma A_j B_j} \right) \cdot 100}$$

where  $A_j$  is the number of individuals of species  $j$ ,  $B_j$  is the number of ponds with species  $j$ , and  $M_j$  is the maturity factor, which is equal to 1.0 when stages above Gosner stage 42 are present. The species was considered dominant when  $I \geq 1.0$ , co-dominant when  $0.01 \leq I < 1.0$ , and subordinate when  $0 < I < 0.01$ . The accuracy of the data was obtained from the values normalized by the transformation  $\text{Log}_{10}(X+1)$ .

For the diversity, the Brillouin Index ( $HB$ ) was used, which is recommended for closed populations (Magurran 1988), like those of a temporary pond.

$$HB = \frac{\ln N! - \sum \ln(n_i)!}{N}$$

where  $N$  is the total number of individuals per sample and  $n_i$  is the total number of individuals of species  $i$ .

Microsoft Excel Professional Plus 2016 was used to calculate the approximations of the factorial numbers, following Lima and Batista (2010). According to Magurran (2011), the Brillouin Index is a robust index that is suitable for application in completely inventoried communities such as those in this study.

**Reference specimens.** The reference specimens were deposited in the Natural History Collection of the Federal University of Piauí (Coleção de História Natural da Universidade Federal do Piauí - CHNUFPI), under

lot numbers CHNUFPI3198 (Hylidae), CHNUFPI3199 (Leptodactylidae), CHNUFPI31200 (Microhylidae), and CHNUFPI31201 (Phyllomedusidae). The collection authorization was emitted by ICMBio (Instituto Chico Mendes de Conservação da Biodiversidade) as authorization number 38966.

## Results

Tadpoles were found in 24 of the 48 sampled ponds, and represented 12 species belonging to four families (Table 1). The total of 403 individuals among the 12 species yielded an  $HB$  of 0.41. The mean abundance per species indicated that *Pseudopaludicola mystacalis* had the greatest abundance (18.8), followed by *Leptodactylus chaquensis* (12.9) and *Physalaemus albifrons* (12.9), while the other species had a mean abundance of  $6.1 \pm 2.7$  (Table 1). This differs from the  $MI$  results, where *Scinax x-signatus* showed the highest  $MI$  (1.0), while all of the other species had  $MI$  values of  $0.55 \pm 0.12$  (Table 1).

The degree of aggregation of tadpoles per species yielded a  $GI$  value of 0.01. Therefore, it is possible to infer that the species exhibited random aggregation behavior without interspecific dependencies.

Based on the occupation of 24 of the 48 ponds sampled, the  $I$  values for the top species were 1.68 for *Leptodactylus chaquensis*, 1.24 for *Physalaemus albifrons*, 1.24 for *Pseudopaludicola mystacalis*, and 1.40 for *Scinax ruber*. Thus, these species were considered dominant while the other species presented an average  $I$  value of  $0.42 \pm 0.23$ , indicating they were co-dominant (Table 1).

The hydroperiod was 29 days, with a mean waterbody retraction of 1.31 mm per day. The hydroperiod predictive model for means between depth and retraction period was established as:  $y = -1.1583x + 32.283$  (Fig. 1).

The distribution of species per pond varied. Ponds 17 and 20 had the largest numbers of species, with seven species each; followed by pond 15, with six species; and ponds 8, 10, 26, and 43, with five species each. The species richness of 11 of the ponds (numbers 4, 5, 9, 23, 27, 31, 34, 37, 41, 42, and 45) ranged from two to four species, and five of the ponds (1, 29, 32, 36, and 48) only had one species each.

The mean temperature of the 48 ponds was  $24.85 \pm 3.78$  °C, dissolved oxygen was  $2.14 \pm 0.42$  mg L<sup>-1</sup>, and the pH was  $5.16 \pm 1.59$ . The substrate was classified in sandy, clayey, and silty, with a depth up to 30 cm. There was no relationship between pond occupation and the abiotic properties according to the coefficients of determination ( $R^2$ , Fig. 2). Although there were no correlations with the abiotic factors, water retraction directly influenced larval survival. Thus, as the waterbody decreased, the proportion of tadpoles between stages 38 and 42 increased (Fig. 3).

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**Table 1.** Species richness and ecological descriptors of the tadpole assemblages. MA – Mean Abundance; MI – Mean Intensity; I – value of Importance; <sup>D</sup> – Dominant / <sup>CD</sup> – Co-dominant species

Family	Common name	MA	MI	I
<b>Hylidae</b>				
<i>Corythomantis greeningi</i> Boulenger, 1896	Greening's Frog	2.35	0.50	0.02 <sup>CD</sup>
<i>Dendropsophus nanus</i> (Boulenger, 1889)	Dwarf Treefrog	7.06	0.67	0.59 <sup>CD</sup>
<i>Dendropsophus soaresi</i> (Caramaschi and Jim, 1983)	Picos Treefrog	8.24	0.57	0.62 <sup>CD</sup>
<i>Scinax ruber</i> (Laurenti, 1768)	Red Snouted Treefrog	11.76	0.60	1.40 <sup>D</sup>
<i>Scinax x-signatus</i> (Spix, 1824)	Venezuela Snouted Treefrog	3.53	1.00	0.34 <sup>CD</sup>
<b>Leptodactylidae</b>				
<i>Leptodactylus chaquensis</i> Cei, 1950	Cei's White-lipped Frog	12.94	0.64	1.68 <sup>D</sup>
<i>Leptodactylus vastus</i> Lutz, 1930	Northeaster Pepper Frog	8.24	0.71	0.82 <sup>CD</sup>
<i>Physalaemus albifrons</i> (Spix, 1824)	Bahia Dwarf Frog	12.94	0.45	1.25 <sup>D</sup>
<i>Pleurodema diplolister</i> (Peters, 1870)	Peters' Four-eyed Frog	5.88	0.40	0.26 <sup>CD</sup>
<i>Pseudopaludicola mystacalis</i> (Cope, 1887)	Cope's Swamp Frog	18.82	0.56	1.25 <sup>D</sup>
<b>Microhylidae</b>				
<i>Dermatonotus muelleri</i> (Boettger, 1885)	Muller's Termite Frog	3.53	0.33	0.33 <sup>CD</sup>
<b>Phyllomedusidae</b>				
<i>Pithecopus nordestinus</i> (Caramaschi, 2006)	No common name	4.71	0.75	0.36 <sup>CD</sup>

## Discussion

In this study, the calculations of *MA* and *MI* did not consider the larval stages, and were based solely on the number of individuals collected during the sample period. The factors affecting survival are both intrinsic and extrinsic (Wilbur 1980; Griffiths et al. 1991; McDiarmid and Altig 1999). The highest *MA* and *MI* values were observed for *P. mystacalis*, *P. albifrons*, and *L. chaquensis*. When assessing the distribution of each of these species, we verified that they present wide adaptability and distribution within Brazil (Frost 2020). For example, *Pseudopaludicola mystacalis* is distributed throughout the Brazilian Cerrado, extending to Bolivia, Paraguay, Argentina, and Uruguay (Frost 2020), illustrating the capacity of this species to adapt to different habitats and climates. *Physalaemus albifrons* occurs in the Brazilian states of Bahia, Minas Gerais, Paraíba, Sergipe, Pernambuco, Ceará, and Maranhão (Bokermann 1966; Langone 2001; Palmeira et al. 2011), demonstrating its capacity to inhabit Piauí under climatic conditions that favor the rapid disappearance of temporary lentic environments. *Leptodactylus chaquensis* occurs in Uruguay, Argentina, Bolivia, and Paraguay (Vaz-Ferreira et al. 1984; Frost 2020), and its occurrence in Brazil has been recorded in the states of Acre, Rondônia, Mato Grosso do Sul, Minas Gerais, São Paulo, Rio Grande do Sul, and Paraná (Frost 2020). Recently Santos et al. (2014) and Lima et al. (2016) expanded its distribution area to include Piauí and Maranhão, which supports our

understanding of the eurytopic nature of *L. chaquensis*.

The temporal unpredictability of ponds causes tadpoles to exhibit dynamic adaptability, forming evanescent communities (Gascon 1993; Alford 1999; Morrison and Hero 2003), and their community structure is related to random environmental factors (Pounds and Crump 1994; Alford 1999). This variability, randomness, and temporal unpredictability concur with our results regarding the degree of community aggregation, which was classified as random. There was no correlative dependence among species with the availability of favorable abiotic conditions. In this study, the requisite condition was water, while the other abiotic variables were not limiting (Fig. 2). Although the other abiotic factors could fulfill an important role (Andrade et al. 2007) in more humid environments, the pressure caused by the scarcity of water plays the dominant role in the xeric Caatinga biome.

The formation of occasional interannual ponds promotes changes in the number and size of such waterbodies, and these stochastic processes are responsible for community instability. This instability is sufficient for species limitation, dispersion, and dominance (Pinto et al. 2006). The dominance of *L. chaquensis* ( $I=1.68$ ), *P. albifrons* ( $I=1.24$ ), *P. mystacalis* ( $I=1.24$ ), and *S. ruber* ( $I=1.40$ ) may be explained by the fact that their adult forms are known for their ubiquity and widespread distribution (Langone 2001; Roberto et al. 2013; Frost 2020). The co-dominance of *D. nanus*, *S. x-signatus*, *P. diplolister*, and *D. muelleri* ( $I=0.40 \pm$

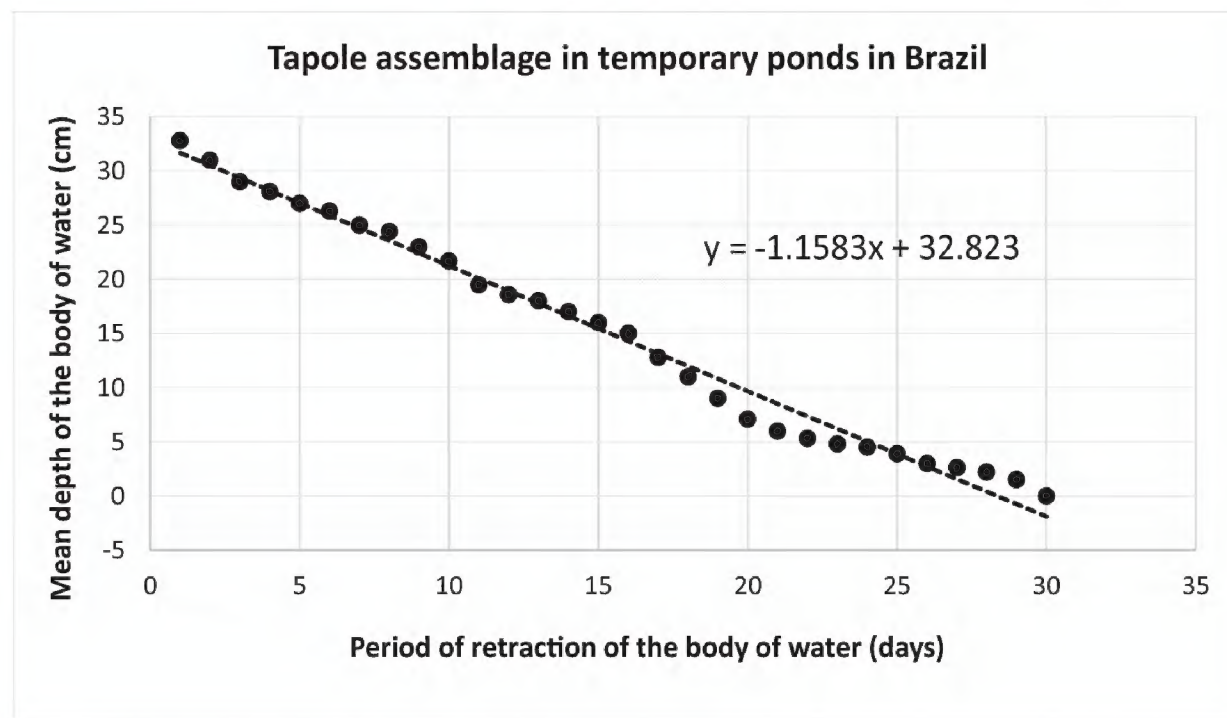


Fig. 1. Hydroperiod, i.e. retraction of the body of water in the study sites.

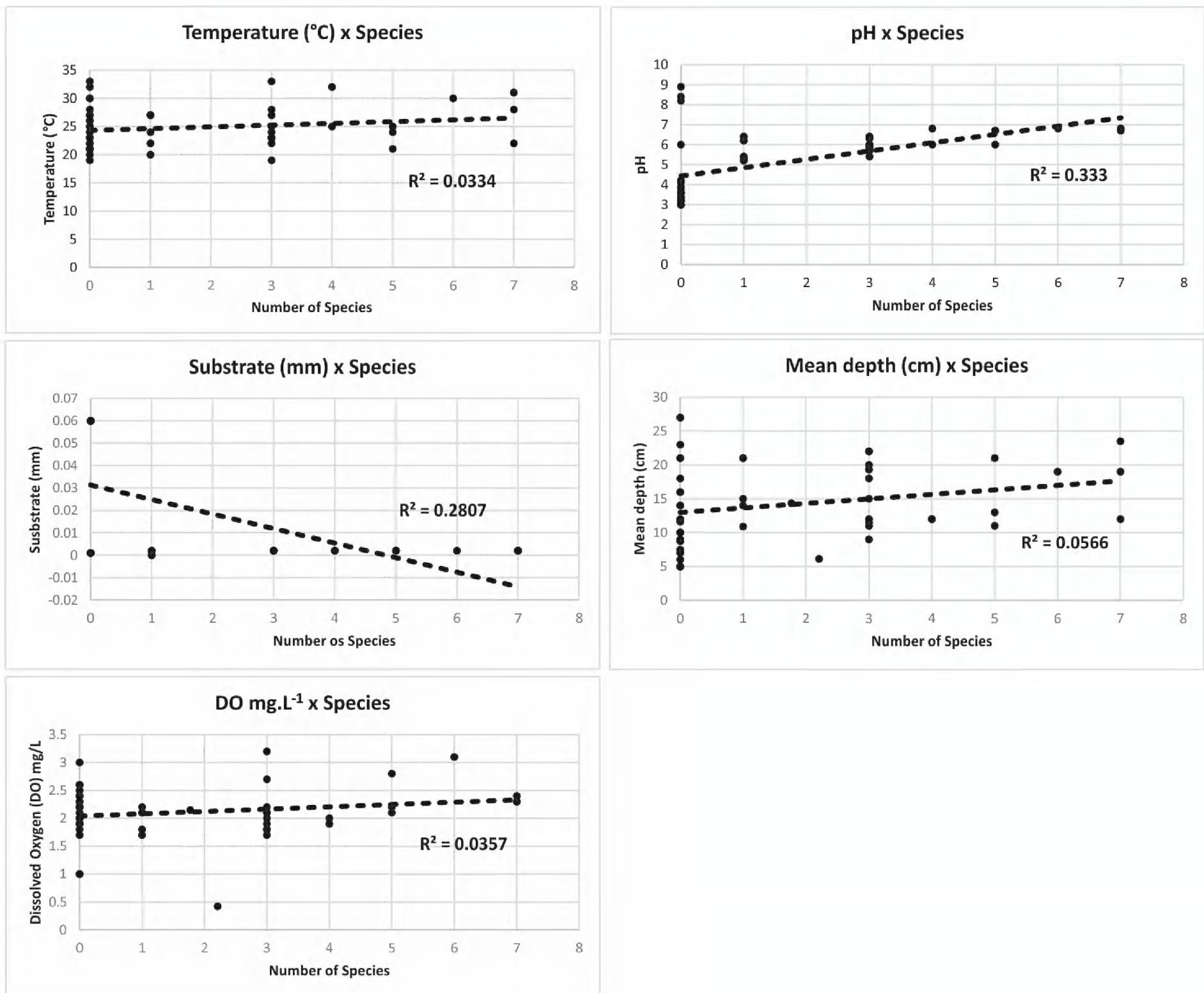
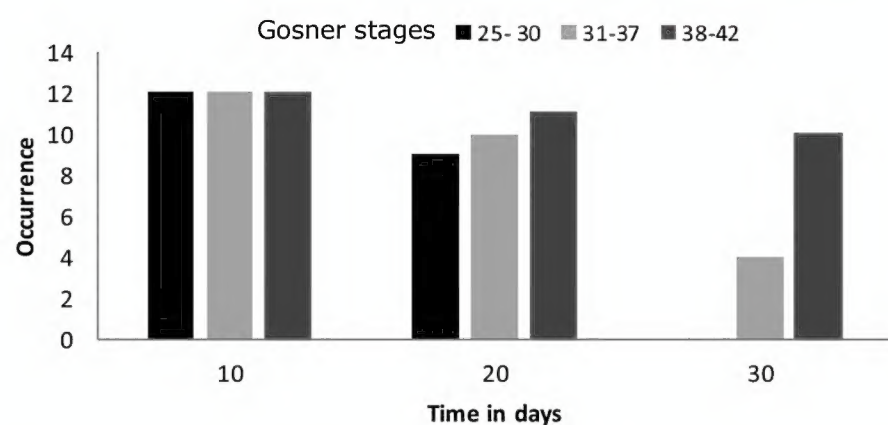


Fig. 2. Coefficients of determination ( $R^2$ ) between occupation of a pond by tadpoles and the various physiochemical water properties. Abbreviation: DO - Dissolved oxygen.

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**Fig. 3.** Hydroperiod and tadpole developmental stages.

0.14) concurs with other studies that have found these species in very different ecosystems, such as Caatinga in the Serra da Capivara (Cavalcanti et al. 2014) and the coast of Parnaíba (Silva et al. 2007; Loebman and Mai 2008). These studies investigated the adult forms, but they do point to dispersion and adaptability in Piauí, which encompasses several different ecosystems (Ab'Saber 2010).

Among the co-dominant species, *Dendropsophus soaresi* occurs in the states of Piauí, Paraíba, Ceará, and Minas Gerais, and its type locality is Picos, which is 300 km from our study area. *Pithecopus nordestinus* is found in the dry Caatinga, which differs from our study area that is comprised of Cerrado with enclaves of anthropized Cocais Forest. Thus, these conditions probably influenced its occupation, resulting in its classification as co-dominant ( $I = 0.58 \pm 0.35$ ). Roberto et al. (2013) reported that *C. greening* was not recorded in any of the 18 municipalities in Piauí which had been inventoried. This corroborates our findings that despite its co-dominance, the index for this species is the lowest among all of the species thus classified ( $I = 0.02$ ). The absence of this species in the findings of Roberto et al. (2013) may be due to its unusual habit of sheltering in rock cracks, in which it covers the exposed cavity with its ossified head (Jared et al. 2005).

The effects of stage and survival have been described by Lima and Pederassi (2012), who pointed out that the success rate of *Rhinella icterica* is associated with the stage of development – i.e., the higher the stage, the greater the developmental success. The results of this study point to the same positive correlation (Fig. 2), as the occurrence of stages 25 to 30 decreased as the water retreated. Protazio et al. (2015) found a similar result by studying tadpole niches, where niche occupation in the semi-arid northeastern Caatinga of Paraíba state did not significantly depend on temperature.

## Conclusions

The successful occupation of xeric environments by frogs is directly related to their capacity to adapt to ephemeral waterbodies, where the main limiting factor is water availability and not the physicochemical properties

of the water. The various specialized adaptations in the developmental dynamics of tadpoles regarding their ability to accelerate metamorphosis in accordance with the reduction of the water body should be further investigated in different biomes and different species, so that we can better understand how these amphibians will adapt to global warming and its aftereffects over the hydrodynamic periods.

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## Tadpole assemblage in temporary ponds in Brazil



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